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BIOLOGICAL APPLICATIONS OF ATMOSPHERIC PRESSURE DIELECTRIC BARRIER DISCHARGES

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ABSTRACT. A reduction of more than 4 orders of magnitude of survivors was obtained by exposing a *Bacillus Stearothermophilus* spores - contaminated surface to an atmospheric pressure DBD post-discharge for 20 minutes. Decontamination mechanisms are investigated assuming that (i) inactivation is obtained when the bacteria DNA is fragmented, (ii) the protein coats are the main protection of the cell core DNA in the case of bacteria spores. The degradation of DNA (plasmid) and protein (RNAse A) samples submitted to the post-discharge is evaluated according to the operating conditions: gas composition, treatment time and sample state, i.e. hydrated or dried samples.

1. INTRODUCTION - CONTEXT

Among the possible alternatives to conventional wet heat treatment, non-thermal plasma technologies can be of interest for the purpose of surface decontamination. Such technologies in gases at pressures below atmospheric pressure (less than 10^3 Pa) have been successfully investigated [1] but in this paper, we will only focus on atmospheric pressure non-thermal plasmas obtained by dielectric barrier discharges in various gas mixtures. A surface decontamination process must be compliant with the following requirements: (i) high efficiency, (ii) low degradation of the treated surface, (iii) reasonable investment costs and (iv) low operating costs. The two last items are satisfied by working at atmospheric pressure. Except in the case of the APGD and jet plume [2,3], non-thermal plasma develops in filaments, with high current density, leading to a local degradation of the surface if directly submitted to the discharge. Furthermore, the treatment shall not be homogeneous. In the present work, there is no direct contact between the plasma and the surface and only the discharge products are transferred to the surface, thus avoiding cathodic sputtering. In such a treatment configuration, significant decontamination efficiencies have been obtained with different bacteria strains under different operating conditions. The present study is an attempt to investigate the inactivation mechanisms occurring during the plasma induced treatment of sporulated bacteria. For this purpose, DNA and protein degradation by the plasma process is studied, assuming that (i) inactivation is obtained when the bacteria DNA is fragmented, (ii) the protein coats are the main protection of the cell core DNA in the case of bacteria spores.

2. EXPERIMENTAL

2.1. Non-thermal plasma source

The non-thermal plasma is obtained by dielectric barrier discharges in a co-axial geometry reactor. The high voltage electrode consists of a metallic rod (2 mm diameter) centered in a Pyrex® tube (6 mm and 3 mm external and internal diameters respectively). A 24 mm length

copper tape covers the external surface of the tube and constitutes the grounded electrode. In this device, discharges propagate in the volume between the high voltage electrode and the internal face of the Pyrex® tube delimited in length by the outer grounded electrode. The DBD reactor is energized by an ac high voltage power supply at 28.5 kHz. Biological samples, placed in a separated box, are submitted to the discharge effluent, 3 cm from the tube outlet. Ozone and nitrogen oxides were detected in gas phase using absorption spectroscopy. H_2O_2 was measured in water droplets exposed to the discharge effluents using a spectrophotometric method. These chemical aspects won't be discussed in the paper. More details can be found in [4].

2.2. Bacteria inactivation evaluation

The bacteria were treated in 10 µL distilled water droplets deposited on sterile glass slides. The suspensions contain 2.10^6 bacteria/mL *Bacillus Stearothermophilus* spores (10 µL giving 2.10^4 bacteria). Droplets could be dried or not before treatment. After treatment, the bacteria on each slide are collected in 2 mL of distilled water. The suspensions obtained are serially diluted and then plated out (100 µL) on an agar surface (Petri dish). After incubation, (36h at 56°C), direct colony counting is done.

2.3. Biochemical techniques

Protein and DNA solutions were treated in 10 µL distilled water droplets (containing 10 µg and 1 µg respectively) deposited on sterile glass slides dried or not before treatment. After treatment, protein and DNA samples were collected respectively in 100 µl and 40 µL distilled water volumes.

Proteins were separated according to their molecular weight by electrophoresis (150V, 90 minutes) on sodium dodecyl sulphate-polyacrylamide gel (SDS-PAGE). Prior to electrophoresis, proteins were submitted to a denaturing treatment.

Agarose gel electrophoresis was used to separate DNA molecules according to their molecular weight and 3-D structure. After migration, the agarose gel was treated by ethidium bromide (DNA intercalant agent), allowing visualization of DNA migration bands exposed to UV illumination. In the case of high DNA degradation, a PCR (Polymerase Chain Reaction) was made on the treated samples to detect the presence of residual DNA molecules.

3. RESULTS –DISCUSION

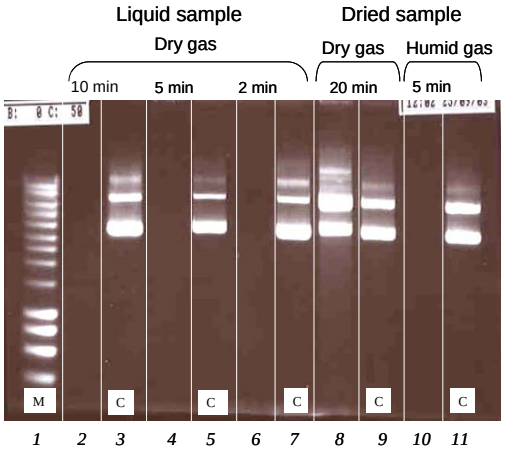
3.1. Bacteria treatment

Using the set-up described in the experimental section, *B. Stearothermophilus* spores were submitted to an air discharge effluent under three different operating conditions: (a) dried spore samples submitted to a wet air discharge effluent (b) liquid spore samples (spores suspended in distilled water) submitted to a dry air discharge effluent (c) dried spore samples submitted to a dry air discharge effluent. Under conditions (a) and (b), the number of survivors was reduced of at least 4 orders of magnitude within 20 minute treatment time whereas the same treatment under conditions (c) led to a population reduction inferior to 90%. From these results, it can be stated that only wet samples (droplets or water film coming from condensation) can be effectively decontaminated using this plasma source. Focusing on the stable oxidative species which can be involved in the inactivation mechanism, two gaseous

products may be considered: ozone and nitrogen oxides (mainly NO_2). The biocidal effect of ozone is well known, and furthermore nitrogen oxide formation in presence of a wet film (or droplets) will lead to an acidification of the solution, through different mechanisms according to the experimental conditions: (i) in gas phase resulting from the OH produced by water dissociation in the discharge (wet air conditions only): $NO_2 + OH \rightarrow HNO_{3\,(g)}$ followed by a dissolution of the HNO_3 into the aqueous phase (Henry constant: 2.1×10^5 M/atm); (ii) in aqueous phase by dissolution of the formed NO_2 (dry and wet air conditions): $2\ NO_2 + H_2O_{\,(l)} \rightarrow HNO_{2\,(aq)} + HNO_{3\,(aq)}$ (Henry constant: 1×10^{-2} M/atm). As a matter of fact, after a 20 minute treatment time of distilled water droplets (pH 6), the pH reached a 1.8 value. Then, under conditions (a) and (b), reactions take place in an acidified liquid phase. This fact should be considered in the following investigations.

3.2. Investigations on DNA

Assuming that bacteria inactivation is obtained if its DNA is irreversibly damaged, a model DNA molecule (plasmid PET9SnI) has been directly submitted to the plasma process. DNA samples were treated with the same operating conditions that led to significant decontamination results on *B. Stearothermophilus* spores (wet samples and dry air discharge). After a 10 minute treatment time, plasmid was no longer detected by electrophoresis gel technique, showing an important degradation / fragmentation of DNA molecules. The same plasmid quantity incubated for 10 minutes in an acidic solution (HNO_3 solution pH 1.8) led to an intact migration band, showing that these pH conditions do not damage DNA molecules. Moreover, when acid formation is prevented by replacing N_2 by argon (no acidification of the wet samples), the migration band disappeared from 2 minute treatment time (Fig. 1 – lanes 6). Nevertheless, a 10 minute treatment time is necessary to obtain a degradation of the total amount of the nucleic acid chains in the sample, as assessed by a negative result of the PCR test. This confirms that oxidative species (O_3 and short lived species) produced by the Ar/O_2 (80/20) discharge are responsible for the DNA degradation.



Lane 1: molecular weight marker (M)
Lanes 3, 5, 7, 9, 11: Controls (C)

Liquid sample / dry Ar/O_2 :
Lane 2: 10 minute treatment time
Lane 4: 5 minute treatment time
Lane 6: 2 minute treatment time

Dried sample / dry Ar/O_2 :
Lane 8: 20 minute treatment time

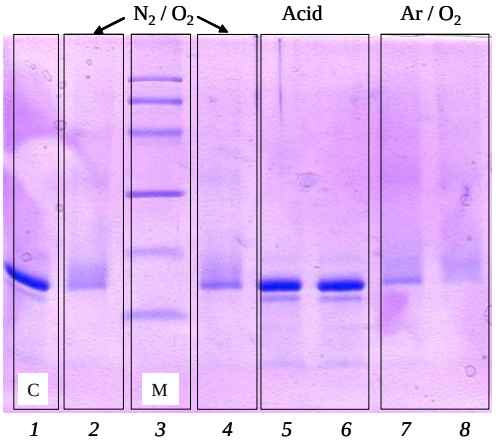
Dried sample / humid Ar/O_2 :
Lane 10: 5 minute treatment time

FIGURE 1. PET9SnI agarose gel electrophoresis of plasmid for different treatments.

Furthermore, the 260 nm absorption band (puric and pyrimidic bases characteristic UV absorption region) exhibited a strong decay correlated with treatment duration, indicating a progressive degradation of DNA bases. The treatment then leads to a fragmentation of the nucleic chain but also to its components (bases) destruction. Figure 1 also shows that, as for spore inactivation, DNA molecules are only significantly damaged when treated in an aqueous phase (droplet solution or condensed liquid film) as illustrated by lanes 8 and 10.

3.3. Investigations on proteins

During bacteria spores treatment, active species must first encounter the protein shell before reaching the DNA molecules in the spore core. The protein chosen to model the protein shell of the spore is the RNAse A. Investigations on the protein were only made on liquid samples and SDS-PAGE migration bands are shown in Figure 2. As for DNA, the incubation of proteins in an acidic solution (pH 1.8) (Figure 2 - lanes 5 and 6) didn't lead to degradation. Moreover, the same weak intensity migration bands were obtained after treatment whether in acidification conditions (air discharge lanes 2 and 4) or in Ar/O_2 conditions (lanes 7 and 8). It can then be stated that acid has a minor role in degradation mechanisms.



Lane 1: Control

Lane 3: molecular weight marker

Lane 2&4: N_2/O_2 discharge treatment

Lanes 5&6: Acid control

Lanes 7&8: Ar/O_2 discharge treatment

FIGURE 2. RNAse A SDS-PAGE for different treatments.

4. CONCLUSION

Degradation of macromolecules such as DNA and proteins, as well as bacteria spore inactivation require wet conditions at least on the sample. For bacteria spore inactivation, the association of acid with oxidizing species is needed whereas DNA and protein degradation only require oxidizing agents. It appears that mechanisms leading to the permeation of the protein shell of the spore are quite more complex than for the degradation of a simple model protein. Note that the pH dependence of the bacteria inactivation is specific to spores, since vegetative bacteria (e. g. *E. Coli*) can be inactivated under non acidic condition [5].

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